PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 19 June 2000 (19.06.00)	
International application No. PCT/US99/25497	**Applicant's or agent's file reference** 794B
International filing date (day/month/year) 29 October 1999 (29.10.99)	**Priority date** (day/month/year) 03 November 1998 (03.11.98)
Applicant BREAKER, Ronald, R. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

10 May 2000 (10.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin d s Colombettes** **1211 G neva 20, Switzerland**	Authorized officer R. Forax
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

US9925497

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C07H 21/00, 21/02, 21/04, C12Q 1/68

A1

(11) International Publication Number: **WO 00/26226**

(43) International Publication Date: 11 May 2000 (11.05.00)

(21) International Application Number: PCT/US99/25497

(22) International Filing Date: 29 October 1999 (29.10.99)

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60/106,829	3 November 1998 (03.11.98)	US
60/126,683	29 March 1999 (29.03.99)	US

(71) Applicant *(for all designated States except US)*: YALE UNIVERSITY [US/US]; Office Of Cooperative Research, 155 Whitney Avenue, New Haven, CT 06520–3886 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: BREAKER, Ronald, R. [US/US]; 71 Hiddlen Land, Guilford, CT 06437 (US). SOUKUP, Garrett, A. [US/US]; Unit 217, 229 Branford Road, North Branford, CT 06471 (US).

(74) Agent: KRINSKY, Mary, M.; 79 Trumbull Street, New Haven, CT 06511–3708 (US).

(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: MULTIDOMAIN POLYNUCLEOTIDE MOLECULAR SENSORS

(57) Abstract

Multidomain polynucleotides responsive to signalling agents are designed and constructed to have at least three domains which can be partially or completely overlapping or nonoverlapping: an actuator (catalytic or reporter) domain, a bridging domain, and a receptor domain. In a typical embodiment, a signalling agent such as a chemical ligand interacts with the receptor domain, which changes conformation or otherwise influences the bridging domain so that the activity, catalytic, or reporter function of the actuator domain is stimulated or inhibited. In some ribozyme embodiments, for example, ligand–specific molecular sensors composed of RNA are created by coupling pre–existing catalytic and receptor domains via novel structural bridges which function such that binding of a ligand to the receptor domain triggers a conformational change within the bridge, and this structural reorganization dictates the activity of the adjoining ribozyme. Processes for allosterically selecting other multidomain polynucleotides typically involve mixing and matching domains to optimize binding or other signal response and/or reporter activity.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/25497

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07H 21/00, 21/02, 21/04; C12Q 1/68
US CL : 435/6.9, 91.31; 536/23.1, 25.1, 25.3

According t International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6.9, 91.31; 436/ 501, 505; 536/23.1, 24.3, 24.31, 24.5, 25.1, 25.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TANG et al. Rational Design of Allosteric Ribozymes. Chemistry and Biology. June 1997, Vol. 4, No. 6, pages 453-459, see entire document, especially Figures 1(B) and 4(A).	1-20
X	WO 98/27104 A1 (YALE UNIVERSITY) 25 June 1998, see entire document, especially page 13, line 10, to page 24, line 2, and Figures 1 and 8(A).	1-20
X	TYAGI et al. Molecular Beacons: Probes that Fluoresce upon Hybridization. Nature Biotechnology. March 1996, Vol. 14, No. 3, pages 303-308, see entire document, including Figures 1 and 2.	1, 4, 8, 10, 12, 14
A,T	SOUKUP et al. Nucleic Acid molecular Switches. Trends in Biotechnology. December 1999, Vol. 17, No. 12, pages 469-476.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 January 2000 (28.01.2000)	10 FEB 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer THOMAS G. IARSON, Ph.D. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/25497

Continuation of B. FIELDS SEARCHED Item 3: WEST data bases (USPT, DWPI, EPAB, JPAB), STN data bases (BIOSIS, CAPLUS, LIFESCI MEDLINE). Search terms: biosensor, polynucleotide, oligonucleotide, RNA, DNA, nucleic acid, ribozyme, aptamer, allosteric, modulate, inhibit, activate, trigger, actuator, receptor, bridge, domain, signal, ligand, R.R. Breaker, G. A. Soukup.

PATENT COOPERATION TREATY

PCT

REC'D 29 JAN 2001

WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

5

Applicant's or agent's file reference OCR-794B	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US99/25497	International filing date *(day/month/year)* 29 October 1999 (29.10.1999)	Priority date *(day/month/year)* 03 November 1998 (03.11.1998)
International Patent Classification (IPC) or national classification and IPC IPC(7): C07H 21/00, 21/02, 21/04; C12Q 1/68 and US Cl.: 435/6.9, 91.31; 536/23.1, 25.1, 25.3		
Applicant YALE UNIVERSITY		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step and industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10 May 2000 (10.05.2000)	05 January 2001 (05.01.2001)
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Joyce Bridges for Thomas G. Larson, Ph.D. Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet)(July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/25497

I. Basis f the report

1. With regard to the **elements** of the international application:*

☒ the international application as originally filed.

☒ the description:
pages 1-51 as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____.

☒ the claims:
pages 52-54 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of _____.

☒ the drawings:
pages 1-19 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____.

☒ the sequence listing part of the description:
pages 1-6 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____.

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained in the international application in printed form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages NONE

☐ the claims, Nos. NONE

☐ the drawings, sheets/~~fig~~ NONE

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*
*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step r industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims NONE	YES
	Claims 1-20	NO
Inventive Step (IS)	Claims NONE	YES
	Claims 1-20	NO
Industrial Applicability (IA)	Claims 1-20	YES
	Claims NONE	NO

2. CITATIONS AND EXPLANATIONS (Rule 70.7)

Claims 1-10 and 12-20 lack novelty under PCT Article 33(2) as being anticipated by Tang et al.

Tang et al. disclose hammerhead ribozymes (Figs. 1 and 4a) comprising a catalytic actuator (ribozyme) domain, a receptor (ATP aptamer) domain where the two domains are joined by a bridging region such that binding of the ligand (ATP) by the receptor domain causes an allosteric change that modulates the catalytic activity of the actuator domain (Figs. 2 and 4b). Tang et al. further disclose (pp. 457-458, bridging paragraph) similar constructs where the ligand for the receptor domain is theophylline and teach that the allosteric ribozymes can be made from DNA (deoxyribozymes). Tang et al. teach that such constructs can be used as biosensors (p. 458, col. 1, 1st full paragraph, last sentence). Tang et al demonstrate that the constructs can be used to detect when the presence of the ligand (Figs. 2 and 4b). Tang et al. disclose methods for preparing the constructs p. 458, col. 2) and teach that combinatorial methods may be used to prepare the constructs (p. 458, col. 1, lns 7-10, and sentence bridging cols. 1 and 2) which would require screening combinatorial libraries for the construct with the desired activities.

Claims 1-20 lack novelty under PCT Article 33(2) as being anticipated by Breaker (WO 98/27104).

Breaker discloses RNA and DNA molecules that can be used as biosenseors (p. 4, lns. 6-12). The biosensor molecules can be used to detect the presence or absence of the compounds (p. 5, lns. 4-7). Breaker discloses an example of a molecule comprising a hammerhead ribozyme domain coupled to an adenosine- or theophylline-binding aptamer domain such that the binding of the adenosine or theophylline ligand modulates the catalytic activity of the ribozyme (p13, lns. 11-23). Breaker teaches the biosensor attached to a solid support (p. 17, ln. 24-p. 18, ln. 16). Breaker teaches a method for producing the biosensor molecules (Example 1, starting p. 19). Breaker also teaches a screening method for selecting biosensor molecules having a desired function (Example 2, starting p. 24).

Claims 1-4, 8, 10-12 and 14-16 lack novelty under PCT Article 33(2) as being anticipated by Tyagi et al.

Tyagi et al. teach a molecular beacon comprising fluorophore and quencing (actuator) domains attached to a hybridizing (receptor) domain by bridging domains (Figs. 1 and 2). When the hybridization domain hybridizes to a target nucleic acid molecule, the binding of the target molecule triggers a conformation change in the hybridizing domain that separates the fluorophore and quenching domains to modulate the activity of the fluorophore domain. Tyagi et al. teach that the molecular beacon can be used to assay the presence or absence or concentration of a specific nucleotide sequence (Figs. 4-7). Tyagi et al. teach an assay using the molecular beacon on a solid support (p. 307, col. 2, 3rd full paragraph). Tyagi et al. teach methods for preparing molecular beacons (pp. 307-308, bridging paragraph).

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/25497

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According to International Patent Classification (IPC) or to both national classification and IPC

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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
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A,T	SOUKUP et al. Nucleic Acid molecular Switches. Trends in Biotechnology. December 1999, Vol. 17, No. 12, pages 469-476.	1-20

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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer THOMAS G. LARSON, Ph.D. Telephone No. (703) 308-0196

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